

WHAT IS CLAIMED IS:

1. A method for determining the effectiveness of a disinfection or sterilization process, said method comprising;

providing a carrier with microorganisms on the carrier;

5 exposing said carrier to the disinfection or sterilization process;

incubating said carrier in a growth medium comprising a first buffer having a first  $pK_a$ , a second buffer having a second  $pK_a$ , and a pH-sensitive dye, wherein said incubating is after said exposing; and

10 determining whether said microorganisms grow in said growth medium during said incubating, wherein the growing of said microorganisms in said growth medium generates acid, thereby changing pH in said growth medium from a first pH within a first pH range to a second pH within a second pH range, wherein said first  $pK_a$  and said second  $pK_a$  are within said first pH range and said second pH range respectively.

15 2. The method of Claim 1, wherein said determining whether said microorganisms have grown comprises determining whether the pH changes in said growth medium from said first pH to said second pH.

20 3. The method of Claim 1, wherein said pH-sensitive dye has a first color in said first pH range and a second color in said second pH range, wherein said determining whether said microorganisms have grown comprises determining whether the dye changes color from said first color to said second color.

4. The method of Claim 1, wherein said system comprises a lower concentration of buffer having a  $pK_a$  in said first pH range than said buffer having a  $pK_a$  in said second pH range.

25 5. The method of Claim 1, wherein said growth medium is contained in an openable enclosure and wherein incubating said carrier in a growth medium further comprises opening the enclosure and immersing said carrier in said growth medium.

30 6. The method of Claim 1, wherein said carrier and said growth medium are located in a container covered with a gas or vapor permeable but microorganism impermeable barrier and wherein exposing said carrier further comprises diffusing a

germicide gas or vapor from outside said container into said container through said barrier.

7. The method of Claim 1, wherein said microorganism comprises a biological indicating microorganism for said disinfection or sterilization process.

5           8. The method of Claim 1, wherein said disinfection or sterilization process comprises a process with a disinfecting or sterilizing agent selected from the group consisting of steam, heat, ethylene oxide, hydrogen peroxide, ozone, chlorine dioxide, peracetic acid, performic acid, formaldehyde, glutaraldehyde, ortho-phthalaldehyde, and hypochlorite salts.

10           9. A self-contained biological indicator comprising;  
a carrier with viable microorganisms on the carrier;  
a container containing said carrier therewithin, wherein at least a portion  
of said container is transparent and wherein said container comprises an opening  
which is covered with a gas or vapor permeable but microorganism impermeable  
15 barrier;  
at least one openable enclosure inside said container, wherein said  
enclosure contains a culture medium which is capable of supporting growth of  
the viable microorganisms;  
a dye which changes color with a change in pH from a first pH range to a  
20 second pH range; and  
a dual buffer system, wherein said dual buffer system comprises a first  
buffer having a first  $pK_a$  and a second buffer having a second  $pK_a$ , wherein said  
first  $pK_a$  and said second  $pK_a$  are within said first pH range and said second pH  
range respectively.

25           10. The self-contained biological indicator of Claim 9, wherein said carrier is selected from the group consisting of a porous substrate, a non-porous substrate, an absorbent substrate, and a non-absorbent substrate.

11. The self-contained biological indicator of Claim 9, wherein said gas or vapor permeable but microorganism impermeable barrier is a nonwoven polyolefin.

12. The self-contained biological indicator of Claim 9, wherein said viable microorganism comprises a biological indicating microorganism for a disinfection or sterilization process.

5 13. The self-contained biological indicator of Claim 9, wherein said openable container comprises a breakable glass ampoule.

14. The self-contained biological indicator of Claim 9, wherein said dye comprises Bromcresol Purple.

15. The self-contained biological indicator of Claim 9, wherein said first buffer comprises at least one phosphate salt.

10 16. The self contained biological indicator of Claim 9, wherein said second buffer comprises at least one acetate salt.

17. The self contained biological indicator of Claim 16, wherein said at least one acetate salt is sodium acetate.

15 18. The self-contained biological indicator of Claim 9, wherein said dual buffer system comprises a lower concentration of buffer having a  $pK_a$  in said first pH range than buffer having a  $pK_a$  in said second pH range.

19. The self-contained biological indicator of Claim 9, further comprising a cap with at least one opening above said barrier, whereby gas or vapor can diffuse into said container through said hole and said barrier.

20 20. The self-contained biological indicator of Claim 9, further comprising a chemical indicator for indicating exposure of said self-contained biological indicator to a disinfection or sterilization process.

21. A culture medium which is capable of supporting growth of viable microorganisms comprising:

25 a nutrient broth;

a dye which changes color with a change in pH from a first pH range to a second pH range; and

30 a dual buffer system comprising a first buffer having a first  $pK_a$  and a second buffer having a second  $pK_a$ , wherein said first  $pK_a$  and said second  $pK_a$  are within said first pH range and said second pH range respectively.

22. The culture medium of Claim 21, wherein said dye comprises Bromocresol Purple.

23. The culture medium of Claim 21, wherein said first buffer comprises at least one phosphate salt.

5 24. The culture medium of Claim 21, wherein said second buffer comprises at least one acetate salt.

25. The culture medium of Claim 24, wherein said at least one acetate salt is sodium acetate.

10 26. The culture medium of Claim 21, wherein said dual buffer system comprises a lower concentration of buffer having a  $pK_a$  in said first pH range than buffer having a  $pK_a$  in said second pH range.